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Ellipsometry of Biofilms

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Ellipsometry of Biofilms

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Degree of Bachelors of Science
by

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Report Submitted To:

Professor Germano S. Iannacchione, Major Advisor

Abstract

The goal of the project was to measure the change of polarization upon reflection and transmission of protein films in order to find the film's thickness and optical constants. In order to accomplish this goal, I needed to find a viable deposition for the thin biofilms. I was able to create a solution using myoglobin, guanidinium hydrochloride, and a sodium phosphate buffer that would allow me to control all aspects of the sample's composition for ellipsometry experimentation in the future.

Acknowledgments

There are many people who have helped me throughout the duration of this project and helped make it the best that it could be. I would like to thank my advisor, Professor Germano Iannacchione, who inspired me to take on this adventurous emerging topic and was there for me at every step of the process. I would also like to thank Margaret Caisse and Jacqueline Malone for ordering the materials I needed for this project and the graduate students in the Order-Disorder Phenomena Laboratory for all of their advice and suggestions. Finally, and most importantly, I would like to thank my family and friends, who provided an immeasurable amount of support throughout this experience.

Executive Summary

Light is described as an electromagnetic wave traveling through space and how the electric field behaves in space is known as polarization. The electric field of a wave is always orthogonal to the propagation direction, so a wave traveling in the z-direction can be described by its x- and y- components. When the light has completely random orientation and phase, it is considered unpolarized. When light has arbitrary amplitude and phase, it is known as being elliptically polarized.

Ellipsometry is a technique for determining the properties of a material from the polarization characteristics of light reflected from its surface. It measures the polarization change is determined by the properties of the sample, such as thickness, uniformity, surface roughness, and refractive index.

The goal of this project is to observe and characterize thin bio-films using spectroscopic ellipsometry in terms of temperature and composition. Since there has been growing interest in the study of proteins, many scientists have begun research in understanding the fundamental physics behind these important structures. Biofilms have also become a central part of a variety of sensors and also have applications in the medical field.

After becoming familiar with the technique, I needed to find a viable deposition for the thin biofilms. Since the ellipsometer measures very small thicknesses and is very sensitive to small changes, the sample's surface must be as uniform as possible. This will prevent any fluctuating data points on the graphs of the polarization change and allow for more accurate data. Also, the ellipsometer has a temperature stage that can be added to observe how the

films change with varying temperature. The samples need to be very consistent in composition so the temperature changes do not produce drastically different results.

I created a solution using myoglobin, guanidinium hydrochloride, and a sodium phosphate buffer that would allow me to control all aspects of the sample's composition for ellipsometry experimentation in the future. I then tried three different ways of applying the solution to glass slides: dipping the slide into the solution, applying the solution using a pipette, and finally pouring the solution onto the slide and using another blank slide to smooth out the surface. As I continued to make solutions and prepare the samples on slides, I would place the previously made slides aside for later comparison to the newer ones. I later noticed that the solutions on the older slides had dried up and formed a crystalline material on the surface of the glass slide. With further investigation, I discovered that the crystal formations were birefringent, meaning that when light hits its surface, it refracts it into two different waves, causing the samples to look prismatic.

In the future, I will be evaluating the myoglobin solution using ellipsometry in order to understand the fundamental physics behind the protein. My hope is that my research will aid future development of thin biofilms and help pave the way for further studies on protein physics

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I. Introduction

The goal of this project is to observe and characterize thin bio-films using spectroscopic ellipsometry in terms of temperature and composition. In recent years, there has been a growing interest in the study of thin films. A thin film is a layer of material that ranges in thickness from a nanometer to several microns. Applications of thin films include semiconductors, optical coatings, storage devices, and biosensors. As the field of biophysics continues expanding, many biologists want to study the physics of proteins, so physicists have been teaming up and using a variety of techniques in order to understand their physical properties.

In order to study these thin biofilms, I first needed to find a viable and controllable deposition for the thin films. I became familiar with the experimental equipment, learned the proper experimental techniques to use on the ellipsometer and practiced sample preparation techniques using myoglobin, a protein that makes meat turn red. Using the analysis program CompleteEASE, I will be able to analyze the data using a variety of techniques, including best fit graphing, to determine the thickness and optical constants of myoglobin. By using the spectroscopic ellipsometer, I hope to understand the fundamental physics of protein thin films. The experiments will allow me to determine a variety of optical constants that will hopefully aid in the future development of biofilms and help pave the way for further studies on protein physics.

II. Light and Polarization

In order to understand ellipsometry, it is important to discuss how a wave's electric field behaves in space and time, which is commonly known as polarization. The electric field of a wave is always orthogonal to the propagation direction. Therefore, a wave traveling in the z-direction can be described by its x- and y- components. When light has completely random orientation and phase, it is considered unpolarized light. For ellipsometry, however, we are interested in the type of electric field that follows a particular path and traces out a distinct shape at any point, known as polarized light. The relative amplitudes of the waves determine the resulting polarization orientation. When two orthogonal light waves are in-phase, the resulting light will be linearly polarized (Figure 1). If the orthogonal waves are exactly out-of-phase and equal in amplitude, the resultant light is circularly polarized (Figure 2). The most common polarization is "elliptical", one that combines orthogonal waves of arbitrary amplitude and phase (Figure 3), which is where ellipsometry gets its name.

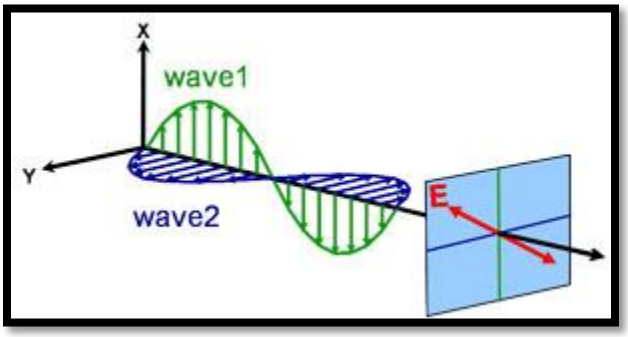

Figure 1: Orthogonal Waves Combined Showing Linear Polarization (J.A. Woollam, 2010)

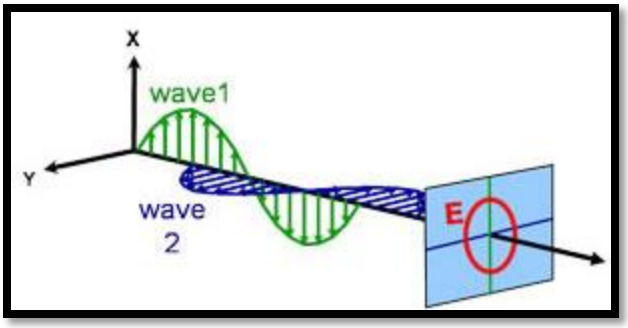

Figure 2: Orthogonal Waves Combined Showing Circular Polarization (J.A. Woollam, 2010)

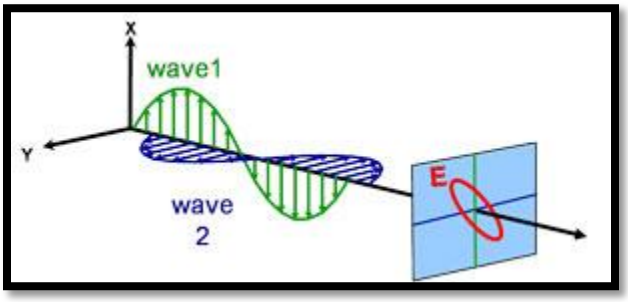

Figure 3: Orthogonal Waves Combined Showing Elliptical Polarization (J.A. Woollam, 2010)

There are two values used to describe the optical properties that determine how light interacts with a material. The complex refractive index ($\tilde{n}$) consists of the index (*n*) and extinction coefficient (*k*) and are related as shown:

$$\tilde{n} = n + ik \quad (1)$$

Alternatively, the optical properties can be written as a complex dielectric function:

$$\tilde{\varepsilon} = \varepsilon_1 + i\varepsilon_2 \quad (2),$$

where $\tilde{\varepsilon} = \tilde{n}^2$ (Tompkins, 2005). The index describes the velocity of light when it travels in a material compared to the speed of light in a vacuum:

$$v = \frac{c}{n} \quad (3),$$

with *c* equal to the speed of light, or 2.998*10^8 m/s.

The speed of light slows down as it enters a material with a higher index. Because the frequency of light waves remains constant, the wavelength shortens. The extinction coefficient describes the amount of energy lost when a wave enters the material. It is related to the absorption coefficient, α, as:

$$\alpha = \frac{4\pi k}{\lambda} \quad (4),$$

where *λ* equals to the wavelength and *k* is the extinction coefficient (J.A. Woollam, 2010). Light also loses its intensity in absorbing materials according to Beer's Law, which states that there is a logarithmic dependence between the transmission of light through a substance and the product of the absorption coefficient and the distance light travels though the material, given as

$$T = \frac{I(z)}{I(0)} = e^{-i\alpha z} \quad (5).$$

From this, we can solve for the new intensity, which is

$$I(z) = I(0)e^{-i\alpha z} \quad (6).$$

Thus, the extinction coefficient relates how quickly light vanishes in a material. These concepts are demonstrated in Figure4, where a light wave travels through two different materials of varying properties before returning to the ambient.

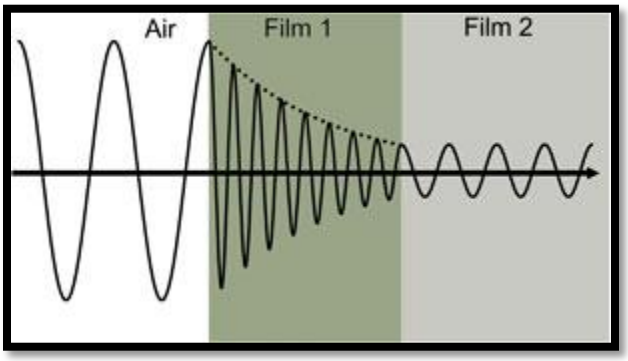

Figure 4: Wave travels from air into absorbing Film 1 and then transparent Film 2. The phase velocity and wavelength change in each material depending on index of refraction (Film 1: n=4, Film 2: n=2) (J.A. Woollam, 2010).

Maxwell's equations need to remain satisfied when light interacts with a material, which leads to boundary conditions at the interface. Incident light will reflect and refract at the interface, as shown in Figure 5. The angle between the incident ray and sample normal will be equal to the reflected angle. Light entering the material is refracted at an angle given by Snell's Law (Tompkins, 2005):

$$n_0 \sin \Phi_i = n_1 \sin \Phi_t \quad (7)$$

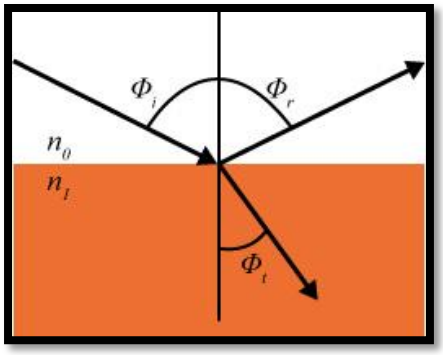

Figure 5: Diagram of light reflecting and refracting (J.A. Woollam, 2010)

The same instance occurs at each interface where a portion of light reflects and the remainder transmits at the refracted angle. This is shown in Figure 6 below. The boundary conditions give different solutions for the electric fields parallel and perpendicular to the sample's surface. Therefore, light can be separated into orthogonal components with relation to the plane of incidence. Electric fields parallel and perpendicular to the plane of incidence are considered *p*- and *s*- polarized, respectively. These components are independent from each other and can be calculated separately. Fresnel described the amount of light reflected and transmitted at an interface between materials (Tompkins, 2005):

$$r_s = \left(\frac{E_{0r}}{E_{0i}}\right)_s = \frac{n_i \cos\Phi_i - n_t \cos\Phi_t}{n_i \cos\Phi_i + n_t \cos\Phi_t} \quad (8)$$

$$r_p = \left(\frac{E_{0r}}{E_{0i}}\right)_p = \frac{n_t \cos\Phi_i - n_i \cos\Phi_t}{n_i \cos\Phi_t + n_t \cos\Phi_i} \quad (9)$$

$$t_s = \left(\frac{E_{0t}}{E_{0i}}\right)_s = \frac{2n_i \cos\Phi_i}{n_i \cos\Phi_i + n_t \cos\Phi_t} \quad (10)$$

$$t_p = \left(\frac{E_{0r}}{E_{0i}}\right)_p = \frac{2n_i \cos\Phi_i}{n_i \cos\Phi_t + n_t \cos\Phi_i} \quad (11)$$

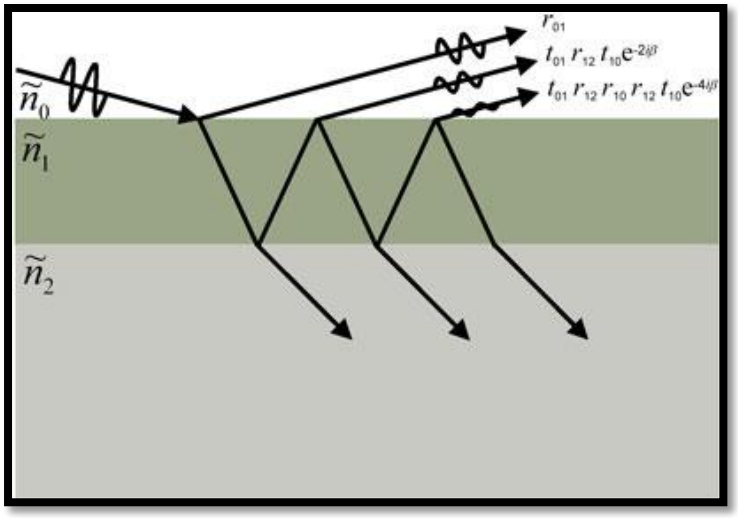

Figure 6: Diagram showing the reflection and refraction of light at different interferences (J.A. Woollam, 2010)

Thin film and multilayer structures involve multiple interfaces, with Fresnel reflection and transmission coefficients applicable at each. It is important to track the relative phase of each light component to determine correctly the overall reflected or transmitted beam. For this purpose, we define the film phase thickness as (J.A. Woollam, 2010):

$$\beta = 2\pi\left(\frac{t_1}{\lambda}\right) n_1 \cos\Phi_1 \quad (12).$$

The superposition of multiple light waves introduces interference that depends on the relative phase of each light wave. Light reflects and refracts at each interface, which leads to multiple beams in a thin film. Interference between beams depends on relative phase and amplitude of the electric fields. Fresnel refection and transmission coefficients can be used to calculate the response from each contributing beam.

III. What is a Protein?

A protein is a biochemical compound that consists of one or more polypeptide folded into either a globular or fibrous form. Many are enzymes that catalyze biochemical reactions and are vital to metabolism. Proteins are composed of small units called amino acids, which are called the building blocks of protein. There are about 20 different amino acids which are commonly known and different proteins are composed of various amino acids put together in varying order with almost limitless combinations. Most proteins are large molecules that may contain several hundred amino acids arranged in branches and chains (Utah State, 1992).

Proteins have a variety of functions based on its type. Some of these types of proteins include (Bailey, 2011):

- Antibodies, which are specialized proteins that are involved in defending an organism's body from antigens,
- Contractile proteins, which are responsible for muscle contraction and movement,
- Enzymes, which facilitate biochemical reactions and are often referred to as catalysts since they speed up chemical reactions in cells,
- Hormonal proteins, which are messenger proteins that help coordinate different bodily activities,
- Structural proteins, which are fibrous and provide support in cells,
- Storage proteins, which store amino acids, and
- Transport proteins, which are carrier proteins that move molecules from one location to another throughout the body.

IV. What is Ellipsometry?

Ellipsometry is a technique used to measure a change in polarization as light reflects or transmits from a material structure. The polarization change is represented as an amplitude ratio, Ψ, and the phase difference, Δ. The measured response depends on the optical properties and thickness of individual materials. Thus, ellipsometry is primarily used to determine film thickness and optical constants. However, it is also used to characterize a material's composition, crystallinity, roughness, and other properties associated with a change in the optical response.

Since the 1960s, as ellipsometry developed to provide the sensitivity necessary to measure nanometer-scale layers used in microelectronics, interest in ellipsometry has grown steadily (J.A. Woollam, 2010). Today, its applications from basic research in physical sciences to semiconductors, flat panel display, biosensors, and optical coatings. This is due to the increased dependence on thin films in a variety of areas and the flexibility of ellipsometry to measure most material types such as dielectrics, semiconductors, metals, superconductors, organics, biological coatings, and composites of materials (J.A. Woollam, 2010).

Ellipsometry is primarily interested in how p- and s- components change upon reflection or transmission in relation to each other. In this manner, the reference beam is part of the experiment. A known polarization is reflected or transmitted from the sample and the output polarization is measured. The change in polarization is the ellipsometry measurement ρ, is written in terms of the amplitude change, Ψ, and phase change Δ as (Tompkins, 2005):

$$\rho = \tan\Psi\, e^{i\Delta} \quad (13).$$

Experimental Set-up

In an ellipsometry experiment, the incident light is linear with both p- and s-components. The reflected light has undergone amplitude and phase changes for both p- and s- polarized light, and ellipsometry measures their changes. The primary tools for collecting ellipsometry data include the light source, polarization generator, sample, polarization analyzer, and detector. The polarization generator and analyzer are constructed of optical components that manipulate the polarization called polarizers, compensators, and phase modulators. Common ellipsometer configurations include rotating analyzer (RAE), rotating polarizer (RPE), rotating compensator (RCE), and phase modulation (PME).

The RAE configuration is shown in Figure 7. A light source produces unpolarized light which is then sent through a polarizer. The polarizer allows light to pass through and is oriented between the p- and s- planes, so both arrive at the sample's surface (J.A. Woollam, 2010). The linearly polarized light reflects from the sample's surface, becomes elliptically polarized, and travels through the analyzer. The amount of light allowed to pass through depends on the polarizer orientation relative to the electric field "ellipse" coming from the sample. The detector converts light to electronic signal to determine the reflected polarization. This information is compared to the known input polarization to determine the polarization change caused by the sample reflection.

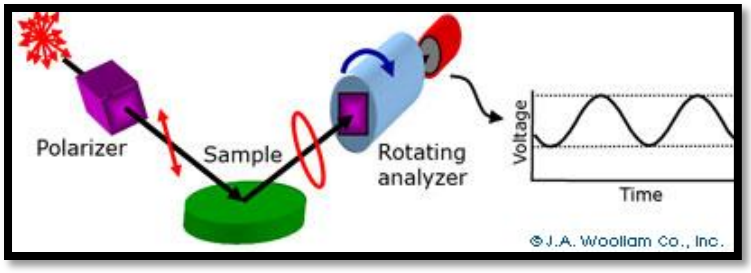

Figure 7: Experimental Set-up for RAE Configuration (J.A. Woollam, 2010)

The ellipsometer I will be using is the J.A. Woollam M-2000® Ellipsometer with Automated Angle capabilities (Figure 8). It is uses the RAE technology to achieve high precision and accuracy. It measures collects over 700 wavelengths ranging from ultraviolet to near-infrared simultaneously. It also measures films from sub-nanometer thickness up to tens of microns and the optical properties from transparent and absorbing materials. This machine also has automatic sample alignment and an attachable temperature control stage used to study how film properties change with respect to temperature and can measure from 4.2K to 873.15K (-268.95 °C to 600 °C) (J.A. Woollam, 2010).

Figure 8: M-2000 ® Ellipsometer

V. Methodology

In order to keep consistency during the ellipsometry experiments in the future, I needed to find a viable and controllable deposition for making the thin biofilms. There are some very important reasons for this necessity:

1. Since the ellipsometer measures very small thicknesses and is very sensitive to small changes, the sample's surface must be as uniform as possible. This will prevent any fluctuating data points on the graphs of the polarization change and allow for more accurate data.
2. The ellipsometer has a temperature stage that can be added to observe how the films change with varying temperature. The samples need to be very consistent in composition so the temperature changes do not produce drastically different results.

The sample making procedure I chose is comprised of three ingredients:

- Myoglobin
- Guanidinium Hydrochloride
- Sodium Phosphate Buffer

Materials

Myoglobin

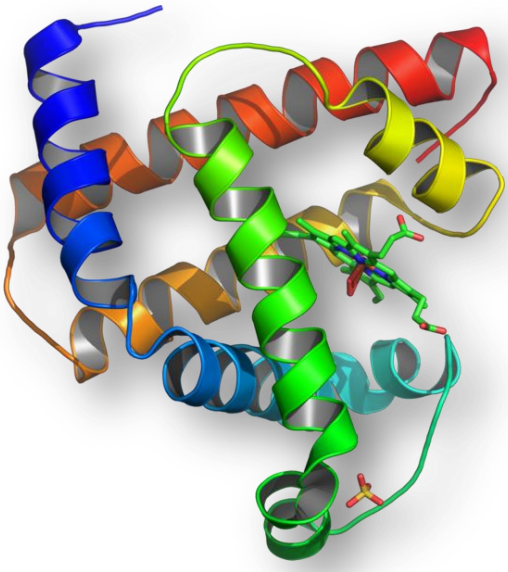

Figure 9: Helical image of myoglobin

Myoglobin (Figure 9) is an iron- and oxygen-binding protein found in the muscle tissue of vertebrates and can be found in almost all mammals. It is related to hemoglobin, which is the iron- and oxygen-binding protein in blood, specifically in the red blood cells. The only time myoglobin is found in the bloodstream is when it is released following muscle injury.

Myoglobin is a single-chain globular protein of 153 or 154 amino acids, containing a heme (iron-containing porphyrin) prosthetic group in the center around which the remaining protein folds, as shown in Figure 11 (Garry, 2004). It has eight alpha helices and a hydrophobic core. It has a molecular weight of 16,700 Daltons, and is the primary oxygen-carrying pigment of muscle tissues (Nelson).

Unlike the blood-borne hemoglobin, to which it is structurally related, this protein does not exhibit cooperative binding of oxygen, since positive cooperation is a property of multimeric/oligomeric proteins only. Instead, the binding of oxygen by myoglobin is unaffected by the oxygen pressure in the surrounding tissue. It is often cited as having an "instant binding tenacity" to oxygen given its hyperbolic oxygen dissociation curve. High concentrations of myoglobin in muscle cells allow organisms to hold their breaths longer. Diving mammals such as whales and seals have muscles with particularly high myoglobin abundance.

For diseases, myoglobin is released from damaged muscle tissue (rhabdomyolysis), which has very high concentrations of myoglobin (Kendrew, 1958). The released myoglobin is filtered by the kidneys but is toxic to the renal tubular epithelium and so may cause acute renal failure. It is a sensitive marker for muscle injury, making it a potential marker for heart attack in patients with chest pain However, elevated myoglobin has low specificity for acute myocardial infarction (AMI) and thus CK-MB, cTnT, ECG, and clinical signs should be of course be taken into account to make the diagnosis.

Guanidinium Hydrochloride

Figure 10: Bottle of Guanidinium Hydrochloride

Guanidinium hydrochloride (Figure 10) is made of guanidine, hydrogen, and chlorine. It is one of the strongest denaturants used in physiochemical studies of protein folding. In 6M guanidinium chloride solution, all proteins with well ordered structure lose it, and most of them become randomly coiled, i.e. they do not contain any residual structure (Lide, 1998). However, at concentrations in the millimolar range in vivo, proteins are shown to change conformation from the prion to non-prion state. This change requires a switch from a beta-sheet based structure to an alpha-helix based structure.

Sodium Phosphate Buffer

Figure 11: Sodium Phosphate Buffer Solution

Sodium phosphate (Figure 11) is a water-based salt solution that is commonly used for maintaining a solution's pH level.

VI. Sample Preparation

During my research, I found that there is very little available on ellipsometry experiments, and almost none that involve biofilms. Many experiments used ellipsometry as a small part of a larger study, so I knew I was going to need to look in other areas for some ways of making a protein solution I could use. After a large amount of searching, I found a procedure that seemed to fit perfectly for an ellipsometry experiment. The procedure is listed below (Georgia Tech, 2007):

- Prepare 5mL of 8M guanidinium hydrochloride solution in dI water.
- Dissolve approximately 9.6mg of myoglobin in 16mL of the sodium phosphate buffer for a 0.6 mg/mL (600 µg/mL) solution.
- Add the reagents in the following order:
  - Buffer
  - Myoglobin
  - Guanidium Hydrochloride
- Leave the sample at room temperature to allow equilibrium to take place before measuring.

Making the Solution

There were a number of observations I made while making the myoglobin solution. First, I mixed the water with the buffer and set it aside. Next, I mixed the myoglobin with the guanidinium hydrochloride. While putting together this mixture, I had expected the solution to be somewhat gel-like, similar to a myoglobin-glycerol mixture I had used previously for a calorimetry experiment. However, it turned out to be an aqueous and clear solution that seemed to have dissolved the myoglobin, as shown in Figure 12.

Figure 12: Mixture of the Myoglobin and phosphate buffer

I had trouble understanding why the myoglobin had dissolved. After some investigation, I learned that since this experimental procedure is popular for protein unfolding experiments, the guanidinium hydrochloride "unraveled" the protein and caused it to appear to dissolve.

Afterwards, I mixed together the buffer solution with the myoglobin-guanidinium solution to make the final solution (Figure 13). The solution was slightly yellow in color and

very transparent. It also appeared to be very aqueous, which caused me to worry about how well it would stay on the slide once applied.

Figure 13: Completed mixture of Myoglobin, Guanidinium Hydrochloride, and Sodium Phosphate buffer

Application of Solution to Glass Slide

My next task was to find the best way to apply the solution to the glass slides. The first method I chose to try was the "dipping" method. This involved dipping the glass slide into the solution and pulling it back out, forming a relatively even coating of the solution on the slide. After a few tries, I determined that this method was not feasible. Since the solution is very aqueous, the solution did not stick to the slide very well and left an extremely thin coating on the slide at best (Figure 14).

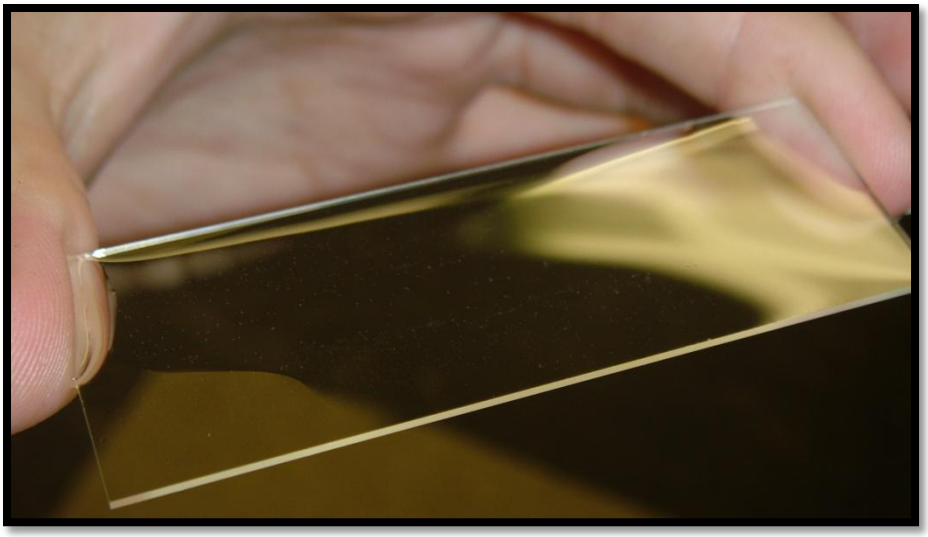

Figure 14: Sample after dipping method was done. From the image, one will notice that the slide does not have much solution on its surface and that more collected on the right end versus being even over the surface.

The second method I tried was putting the solution onto the slide's surface using a pipette. This method would allow me to control how much solution was out onto the slide and would theoretically let me create a uniform surface on the slide. In comparison to the dipping method, the pipette method definitely allowed for more control over the deposition of the solution onto the slide, but did pose some problems. It was not the best way of controlling how much solution was on the slide because in order to cover the slide fully, I needed to put quite a bit of the solution on it. The pipette also was likely to cause air bubbles on the slide, making it non-uniform, as shown in Figure 15 below.

Figure 15: Sample prepared using the pipette method. As stated, one of this method's flaws is that it can cause air bubbles on the surface of the slide, which could cause fluctuations in the ellipsometry data.

The final method I tried was one suggested to me by some of the Physics graduate students. They suggested that I apply the solution to the slide then use another blank slide to smooth out the solution to make it uniform across the slide's surface. This would control both the amount of solution on the slide and the solution's uniformity. As it turned out, this method proved to be the best method for making the slides. As shown in Figure 16, the slide appeared to not have any deformities or obstructions on the surface, which is important since due to the ellipsometer's sensitive measurements, and is uniform across the entire slide.

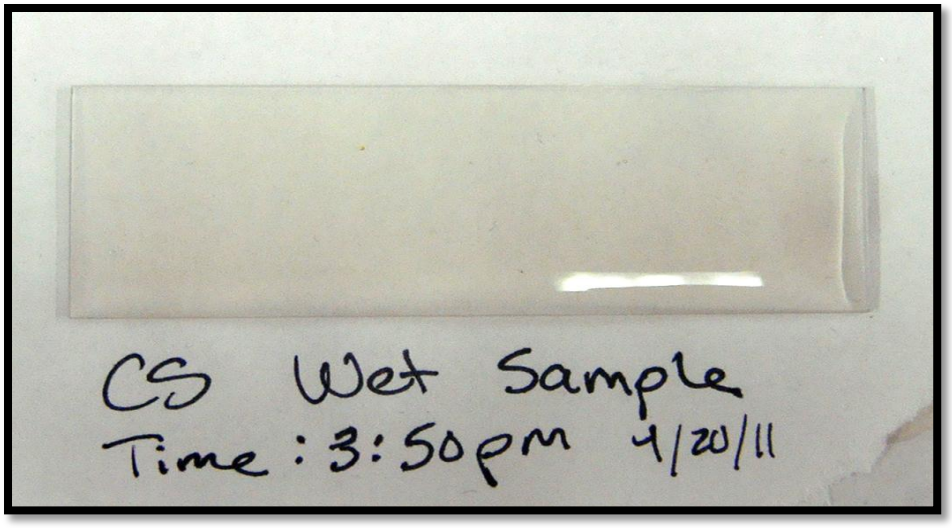

Figure 16: Sample using the slide smoothing technique to ensure a smooth application and uniform surface. If the surface was rough, the data collected from the ellipsometry experiments would have fluctuations where the surface changes.

VII. Results

As I continued to make solutions and prepare the samples on slides, I would place the previously made slides aside for later comparison to the newer ones. After some time, I went back to place my newer slides with the older ones and noticed something very interesting that had happened to the older samples.

When I went back to my samples, I saw that they had formed some kind of a salt-like residue on the slides from them drying up over time. After a closer look at them, the solution had actually formed a crystallized pattern (Figure 17).

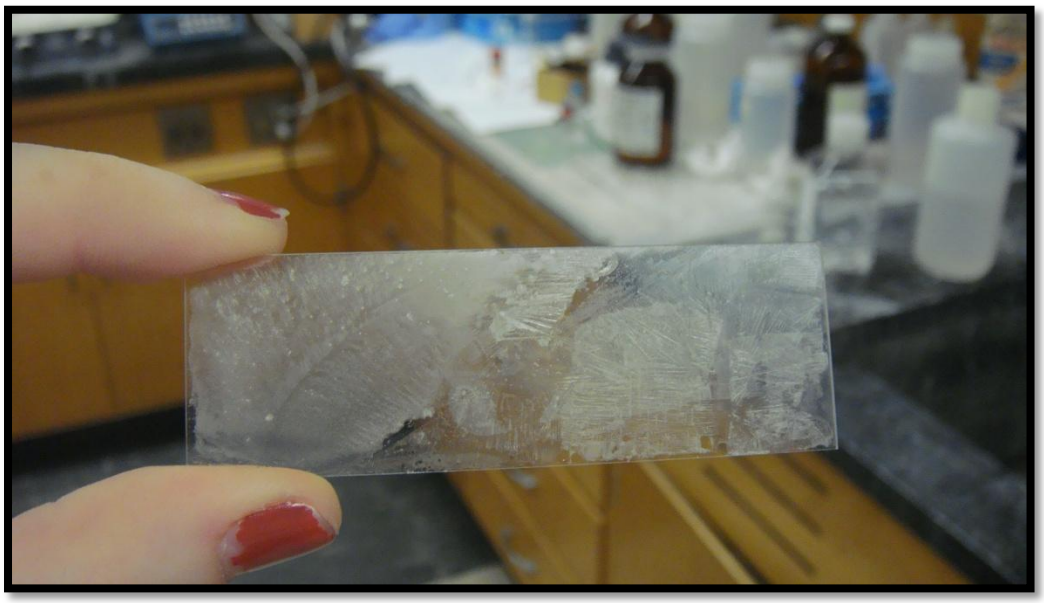

Figure 17: First image of a crystallized sample.

I decided to see if the crystallization patterns were at all affected by the surface of the glass slides, so I took a paper towel and wiped a few of the slides' surfaces down in different

directions. Unfortunately, from what I could tell, the roughness and pattern of the slide's surface do not seem to overly affect the pattern of crystallization on the slide (Figure 18).

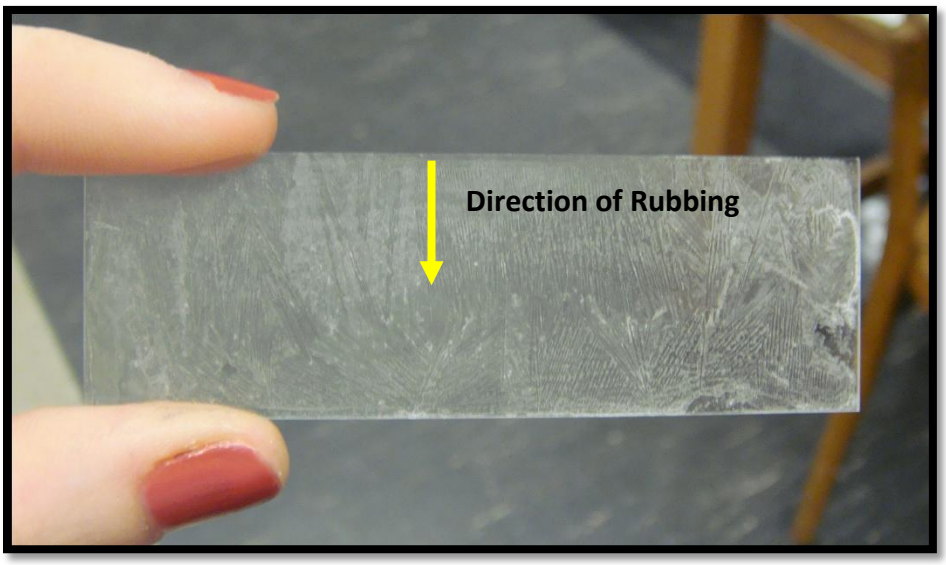

Figure 18: Crystallized sample using a slide that was rubbed down vertically from the top of the slide to the bottom twice with a KimWipe©.

Since this change came as an unexpected, but exciting surprise, I worked to see if these crystallized slides would be useful for experimentation. The slides' surfaces were not perfectly uniform after the crystallization occurred, so I looked at the slides under a microscope to see what the crystalline structure looked like. When I looked through the eyepiece at the sample, the crystal structures were reflecting the light from the source, and looked prismatic (Figure 19).

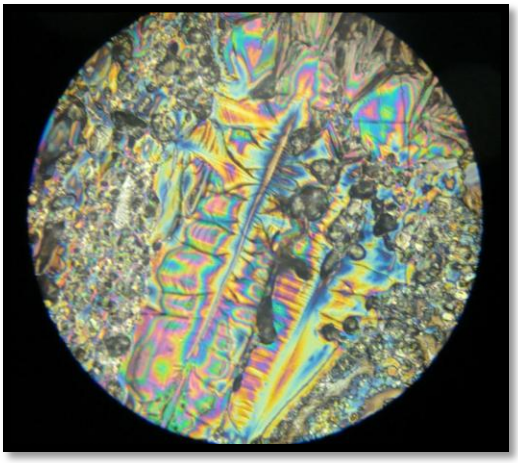

Figure 19: Microscope image of crystallized slide. The light from the microscope's light source reflected from the sample giving the prism effect in the image.

The prismatic effect off the crystals is due to the material exhibiting birefringence. Birefringence occurs when a material has different indexes of refraction for different directions of polarization. When the sample is oriented in unpolarized light, its refractive index could be one value. When that same sample is oriented slightly differently, the refractive index changes value, which causes the color changes across the sample (Figure 20).

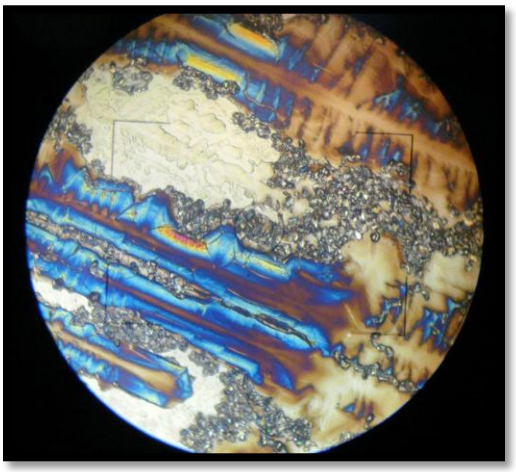

Figure 20: Image of the same crystallized sample rotated 90°. The colors on the sample change upon rotation due to birefringence.

The reason the samples form crystals is in the solution's composition. The sample composition I chose forms a solution that is commonly used in dichromism experimentation. Dichromism (also referred to as pleochromism) is used to describe materials that cause visible light to split into beams with different wavelengths, or a material in which light that has different polarizations is absorbed by different amounts. In crystals, the strength of the dichroic effect depends strongly on the wavelength of light hitting them, causing the different colors when viewed. This technique is commonly used in polarized filters and sunglasses lenses. Since the samples were aqueous solutions, I would not have noticed any crystalline properties of the solution until they dried.

With this new development, I hope to test both the aqueous solution and the new crystallized samples on the ellipsometer. I can then compare the wet samples' results to the crystallized samples, allowing me to further understand the physical properties of the solution, including the thickness and varying optical constants.

VIII. Conclusion

The main goal of this project is to study the behavior of thin biofilms as a function of composition and temperature using ellipsometry. In order to begin, I first needed to find a process of making a thin protein film that will allow me to have control over the sample's initial composition. I was able to create a deposition for the protein film that allows me to have complete control of its composition. As an added bonus, I discovered that the solution, when left to dry, forms crystals on the slides, which turned out to be birefringent. This new discovery will allow me to get a better understanding of the solution's optical properties during experimentation.

In the future, I will be evaluating the myoglobin solution using ellipsometry in order to understand the fundamental physics behind the protein. The study of thin biofilms is an emerging topic among scientists, and they have recently become interested in how the proteins work physically. Thanks to this growing interest, physicists have begun working to learn and understand the fundamental physics of proteins. Biofilms have also become a central part of a variety of sensors. My hope is that my research will aid future development of thin biofilms and help pave the way for further studies on protein physics.

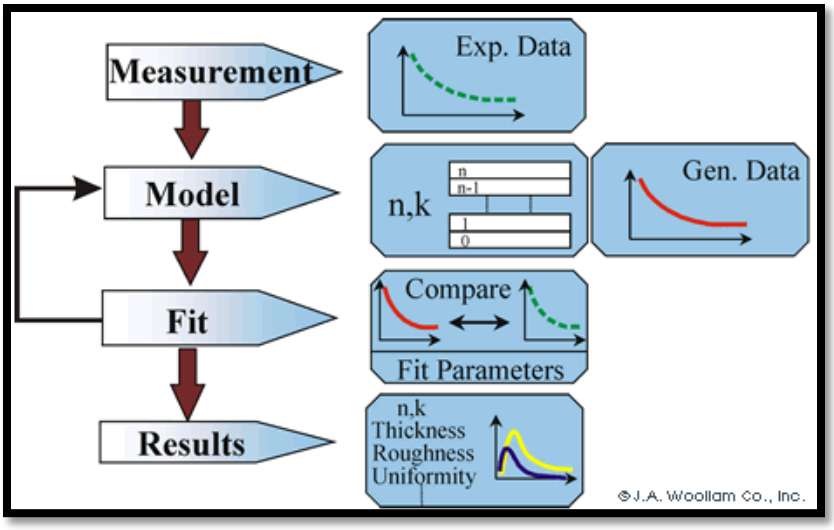

Figure 21: A table for understanding the process of ellipsometry data analysis (J.A. Woollam, 2010).

After evaluating the samples using the spectroscopic ellipsometer, I will be analyzing the data using the CompleteEASE modeling program. This program will allow me to compare my experimental data to a model of how the data should look. From there, I will be able to fit my model and compare it to my measurements in order to determine the sample's thickness, optical constants, roughness, and uniformity.

Bibliography

Tompkins, Harland G., and Irene, Eugene A. (2005). *Handbook of Ellipsometry*. Norwich, NY: William Andrews Inc.

Nelson DL, Cox MM (2005). *Lehninger's Principles of Biochemistry*(4th ed.). New York, New York: W. H. Freeman and Company.

Bailey, Regina. "Protein Function." *Biology*. N.p., n.d. Web. 28 Apr. 2011. <http://biology.about.com/od/molecularbiology/a/aa101904a.htm>

"Spectroscopic Ellipsometry Tutorial: Introduction - J.A. Woollam Co." *Spectroscopic Ellipsometers - J.A. Woollam Co*. N.p., n.d. Web. 7 Feb. 2011. <http://www.jawoollam.com/tutorial_1.html>.

"Spectroscopic Ellipsometry Tutorial: Light and Materials I - J.A. Woollam Co."*Spectroscopic Ellipsometers - J.A. Woollam Co*. N.p., n.d. Web. 15 Feb. 2011. <http://www.jawoollam.com/tutorial_2.html>.

"Spectroscopic Ellipsometry Tutorial: Light and Materials II - J.A. Woollam Co."*Spectroscopic Ellipsometers - J.A. Woollam Co*. N.p., n.d. Web. 15 Feb. 2011. <http://www.jawoollam.com/tutorial_3.html>.

"Spectroscopic Ellipsometry Tutorial: Ellipsometry Measurements - J.A. Woollam Co."*Spectroscopic Ellipsometers - J.A. Woollam Co.* N.p., n.d. Web. 23 Feb. 2011. <http://www.jawoollam.com/tutorial_4.html>.

Garry, G. A. (2004). George A. Ordway and Daniel J. Garry. *Journal of Experimental Biology*, 3441-3446.

H. Jansson, J. S. (2010). The protein glass transition as measured by dielectric spectroscopy and differential scanning calorimetry. *Biochimica et Biophysica Acta*, 20-26.

Hippel, A. V. (1954). Dielectric Materials and Applications. *Technology Press of MIT*.

Kendrew, J. (1958). A Three-Dimensional Model of the Myoglobin Molecule Obtained by X-Ray Analysis. *Nature*, 181-186.

Lide, David R. (1998). *Handbook of Chemistry and Physics* (87 ed.). Boca Raton, FL: CRC Press. pp. 3–296.

Georgia Tech (2007). Protein Unfolding Equilibrium by Circular Dichroism. *School of Chemistry & Biochemistry*. Atlanta, GA